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Stricter regulations on volatile organic compounds (VOCs) and hazardous air pollutants (HAPs) have increased the demand for more efficient abatement technologies. One promising technology for removing VOCs from polluted gas streams is biofiltration, a process in which contaminated air is passed through a biologically active bed. Although bioreactors containing bacteria have been employed to treat waste streams contaminated with VOCs, they are generally only practical for removing low concentrations of VOCs. In this study, a strain of the dimorphic black yeast *Exophiala lecanii-corni*, which has been shown to degrade high concentrations of toluene in a bioreactor, was evaluated for its ability to degrade a variety of VOCs, both individually and in mixtures. The fungus was found to be able to use toluene, ethyl benzene, methyl propyl ketone, n-butyl acetate, and ethyl ethoxypropionate as sole sources of carbon and energy; however, *E. lecanii-corni* was unable to degrade either xylene or benzene. When mixtures of chemicals were added to cultures of *E. lecanii-corn,* the fungus again degraded all of the chemicals except xylene and benzene. In separate experiments to determine the effects of nitrogen availability and pH on the fungus' degradation ability, it was observed that VOC degradation was inhibited when no supplemental nitrogen was added to the nutrient medium and when the pH of the medium was less than 3. These preliminary studies indicate that *E. lecanii-corni* would be a feasible organism to use in biofiltration due to its ability to degrade a wide range of VOCs, even under harsh environmental conditions.

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Use of the Fungus *Exophiala lecanii-corni* to Degrade a Mixture of VOCs

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Abstract

Stricter regulations on volatile organic compounds (VOCs) and hazardous air pollutants (HAPs) have increased the demand for more efficient abatement technologies. One promising technology for removing VOCs from polluted gas streams is biofiltration, a process in which contaminated air is passed through a biologically active bed. Although bioreactors containing bacteria have been employed to treat waste gas streams contaminated with VOCs, they are generally only practical for removing low concentrations of VOCs. In this study, a strain of the dimorphic black yeast *Exophiala lecanii-corni*, which has been shown to degrade high concentrations of toluene in a bioreactor, was evaluated for its ability to degrade a variety of VOCs, both individually and in mixtures. The fungus was found to be able to use toluene, ethyl benzene, methyl propyl ketone, n-butyl acetate, and ethyl ethoxypropionate as sole sources of carbon and energy; however, *E. lecanii-corni* was unable to degrade either xylene or benzene. When mixtures of chemicals were added to cultures of *E. lecanii-corni*, the fungus again degraded all of the chemicals except xylene and benzene. In separate experiments to determine the effects of nitrogen availablity and pH on the fungus' degradation ability, it was observed that VOC degradation was inhibited when no supplemental nitrogen was added to the nutrient medium and when the pH of the medium was less than 3. These preliminary studies indicate that *E. lecanii-corni* would be a feasible organism to use in biofiltration due to its ability to degrade a wide range of VOCs, even under harsh environmental conditions.

Introduction

Over the past twenty years, the use of biological air pollution control has become a popular treatment alternative for contaminated gas streams. The use of biofiltration has worked particularly well for processes that emit a steady gas stream containing low concentrations of one or two contaminants[1]. In recent years, however, there has been a drive to expand the use of biofiltration to applications that emit a variable, complex mixture of chemicals in the off gas because of the potentially high cost of operating more traditional chemical-physical treatment options, and the desire to achieve higher removal efficiencies than these traditional treatment options can sometimes offer[2–6].

Recent studies have shown that it can be difficult to obtain consistent removal efficiencies in biological treatment systems that are treating a mixture of volatile organic compounds (VOCs). Problems with these systems include the inability to maintain constant operating conditions, such as moisture content and nutrient availability; loss of microbial populations that can degrade the contaminants of interest; and inhibition of microbial activity by one or more of the chemicals in the gas stream[2–6].

In a majority of the studies conducted to date, the biofiltration systems intended to treat mixtures of VOCs have been inoculated with mixed microbial cultures, most of which have consisted primarily of bacteria. However, it is often difficult to maintain these original culture conditions within the bioreactor, especially as environmental and operating conditions change over time. In this study we propose an alternative approach to inoculating a biofiltration system to treat a mixture of VOC contaminants.

Fungal biofilters have been shown to be extremely efficient at degrading a variety of VOCs, such as toluene, styrene, and methyl ethyl ketone, even when the biofilters were operated under harsh environmental conditions[7–10]. Also, in many of these studies, the fungi have displaced the original bacterial population present in the system, indicating that they are hardy competitors and are unlikely of being replaced by another culture that could be introduced into the system. These qualities indicate that fungal bioreactors may be an attractive

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alternative for treating VOC mixtures. Therefore, we are developing a bioreactor inoculated with the fungus *E. lecanii-corni* that is capable of degrading multiple contaminants.

Our main objectives of this study include 1) determining the ability of a culture of *Exophiala lecanii-corni* to degrade a variety of VOCs emitted from industrial operations, 2) investigating the possible inhibition of *E. lecanii-corni* when the culture is exposed to a mixture of the selected VOCs, and 3) determining the range of environmental conditions that the culture can withstand before the degradation efficiency of VOCs decreases in a vapor phase bioreactor.

Materials and Methods

Chemicals

VOCs: Toluene, ethyl benzene, benzene, *p*-xylene, n-Butyl acetate, methyl propyl ketone, and ethyl ethoxypropionate

Basal medium: distilled water, which contained the following compounds (g/ℓ): $(NH_4)_2SO_4$ 1.0, $MgSO_4 \cdot 7H_2O$ 0.25, $CaCl_2 \cdot 2H_2O$ 0.02, $CuCl_2 \cdot 2H_2O$ 0.17×10^{-3}, $CoCl_2 \cdot 6H_2O$ 0.24×10^{-3}, $ZnSO_4 \cdot 7H_2O$ 0.58×10^{-3}, $MnSO_4 \cdot H_2O$ 1.01×10^{-3}, $Na_2MoO_4 \cdot 2H_2O$ 0.24×10^{-3}, $NiCl_2 \cdot 6H_2O$ 0.10×10^{-3}, and $FeSO_4 \cdot 7H_2O$ 1.36×10^{-3}

Assay Preparations

Cultures for the screening were prepared by cultivating cells from a pure culture of *Exophiala lecanii-corni* growing on toluene as its sole source of carbon and energy. The cells were centrifuged and washed with fresh, sterile basal medium twice. The cells were resuspended in 50 mℓ of fresh, sterile basal medium and then blended for 30 s in a Waring Blendor.

Degradation Assay. One (1.0) mℓ aliquots of the blended culture were transfered to a 160-mℓ serum bottle containing 9 mℓ of sterile basal medium. The bottles were sealed with a butyl rubber septum and crimp cap. For the single chemical cultures, a stoichiometric amount of the target chemical (19 μmol C/10 mℓ) was then added as a neat liquid using a sterile syringe. For the cultures given a BTEX mixture, 9.5 μmol C/10 mℓ of each chemical was added. For cultures given a paint VOC mixture, 19 μmol C/10 mℓ of methyl propyl ketone and 9.5 μmol C/10 mℓ each of toluene, xylene, ethyl ethoxypropionate and n-butyl acetate were added. Triplicate cultures were made for each chemical or chemical mixture. Duplicate, killed controls were also made for each chemical or chemical mixture. The cultures were stored on a shaker table at 23°C. The headspace of the bottles was sampled using a gas chromatograph (GC) on a regular basis to determine the mass of chemical degraded with time.

Nitrogen Assay. One (1.0) mℓ aliquots of the blended culture were transfered to a 160-mℓ serum bottle containing 9 mℓ of sterile basal medium, with concentrations of $(NH_4)_2SO_4$ ranging from 0-10 g/L. The bottles were sealed with a butyl rubber septum and crimp cap. Toluene (19 μmol C/10 mℓ) was then added as a neat liquid using a sterile syringe. Triplicate cultures were made for each $(NH_4)_2SO_4$ concentration tested. Duplicate, killed controls were also made. The cultures were maintained under stationary conditions at 23°C. The headspace of the bottles was sampled using a gas chromatograph (GC) on a regular basis to determine the mass of chemical degraded with time.

pH Assay. Five (5.0) mℓ aliquots of the blended culture were transfered to a 250-mℓ bottle containing 45 mℓ of sterile basal medium, with pHs ranging from 2.0-4.2. The bottles were sealed with a Teflon-lined, Mininert valve screw cap. Toluene (19 μmol C/10 mℓ) was then added as a neat liquid using a sterile syringe.

Triplicate cultures were made for each pH tested. Triplicate killed controls were also made. The cultures stored on a shaker table at 23°C. The headspace of the bottles was sampled using a gas chromatograph (GC) on a regular basis to determine the mass of chemical degraded with time. Cell yield was determined by measuring the increase in dry weight of the fungal culture as a function of toluene mass degraded during the experiment.

Analytical Methods

VOC Degradation Measurements. The amount of toluene present in the headspeace of the vials was determined by injecting 0.5-mℓ gas samples onto a Hewlet Packard (HP, Palo Alto, CA, USA) Model 6890 Gas Chromatograph (GC) equipped with a flame-ionization detector (FID) and a HP-5 capillary column. Ultra high purity (UHP) helium was used as the carrier gas at a flow rate of 16 mℓ/min. The FID was supplied with 28.4 mℓ/min UHP helium, 50 mℓ/min UHP hydrogen, and 450 mℓ/min zero grade air. The FID was maintained at 250°C. The column was operated at 50°C for 2 min, increased to 140°C at a rate of 12°C/min, and maintained at 140°C for 3 min.

Dry Weight Measurements. An aluminum planchette and Whatman filter paper (0.2 μm) were dried in a 105°C oven for 4 h, cooled to room temperature in a desicator, and then weighed on a four-place balance. The *E. lecanii-corni* assay culture was filtered through the dried Whatman filter paper using a vacuum filter apparatus, which was replaced into the planchette and dried in a 105 °C oven for 4 h. The filtered culture was then cooled to room temperature in a desicator and weighed.

Results

Results indicate that *Exophiala lecanii-corni* was able to degrade repeated additions of toluene, ethyl benzene, n-butyl acetate, and methyl propyl ketone in batch cultures, using each as a sole carbon and energy source. The degradation of the above chemicals was confirmed by an observed increase in turbidity in the cultures. *E. lecanii-corni* was also able to use ethyl ethoxypropionate as a sole source of carbon and energy. Since the ethyl ethoxypropionate was so soluable, headspace concentrations could not be determined directly using the GC method. Instead, growth on ethyl ethoxypropionate was determined by monitoring the increased cell mass of the cultures compared to that of the killed controls. In cultures to which benzene and p-xylene were added, the loss of the target chemical in the viable culture mirrored that of the killed control. This result, along with the observation that there was no visible increase in turbidity in the viable cultures as compared to the killed controls, indicate that *E. lecanii-corni* was unable to use benzene and p-xylene as sole sources of carbon and energy.

To determine if p-xylene and benzene could be degraded by cometabolic processes, or if the presence of these chemicals would inhibit growth of *E. lecanii-corni* on other chemicals, mixtures of the target chemicals were added to cultures of *E. lecanii-corni.* The results from these studies are shown in Figures 1 and 2. In the cultures grown on a BTEX mixture, the toluene and ethyl benzene were degraded, but the p-xylene and benzene were not. These results indicate that the enzymes produced by *E. lecanii-corni* to degrade toluene and ethyl benzene do not fortuitously degrade p-xylene and benzene. Also, the results indicate that the presence of p-xylene and benzene does not inhibit degradation of toluene or ethyl benzene.

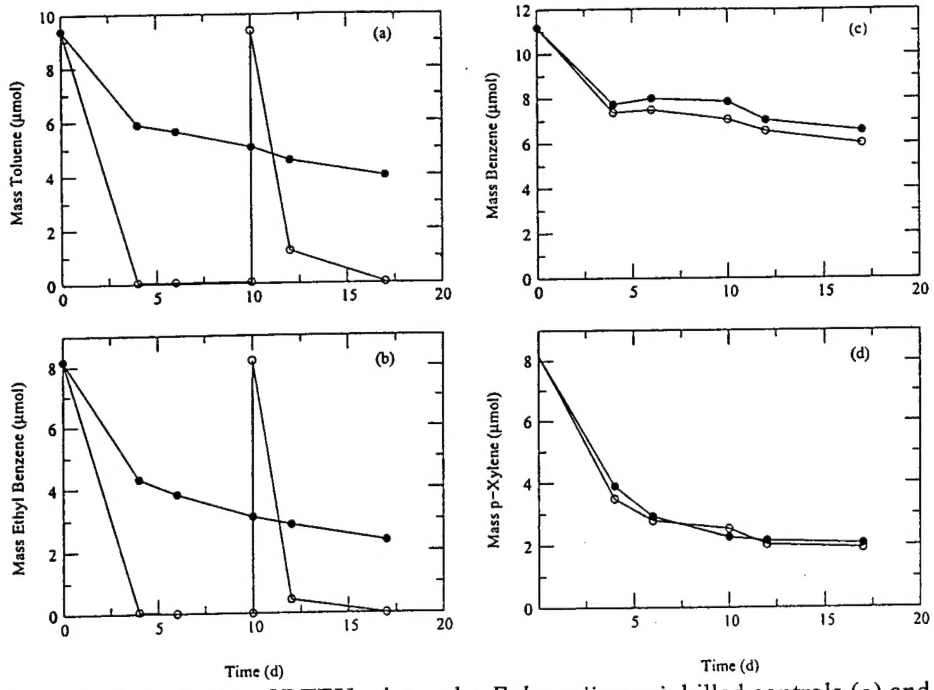

Figure 1. Degradation of BTEX mixture by *E. lecanii-corni*: killed controls (•) and viable cultures (o).

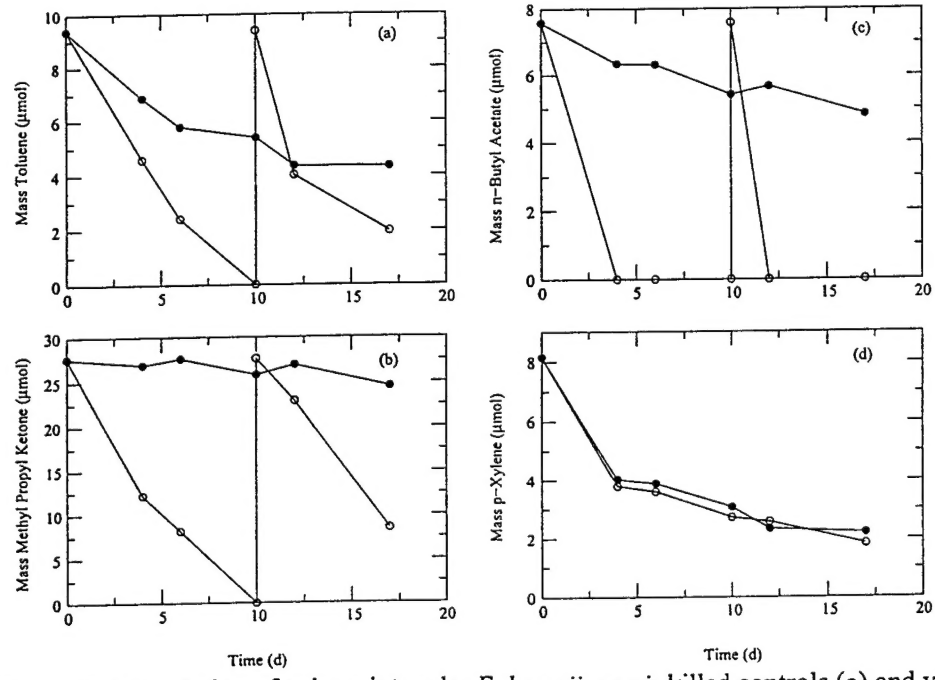

Figure 2. Degradation of paint mixture by *E. lecanii-corni*: killed controls (•) and viable cultures (o).

In the cultures grown on a paint VOC mixture (Figure 2), p-xylene, again, was not degraded. The presence of p-xylene, however, did not inhibit the degradation of the other chemicals. The results also suggest that n-butyl acetate was degraded preferentially over toluene and methyl propyl ketone. Again, the amount of ethyl ethoxypropionate in the culture was not determined, but it was assumed that it was degraded based on the results obtained from the cultures given ethyl ethoxypropionate only. This will be confirmed in subsequent column studies.

In the nitrogen assays, it was observed that at least 0.01 g/ℓ of $(NH_4)_2SO_4$ was needed in order to achieve repeated degradation of toluene additions (data not shown). The culture that was not given any supplemental nitrogen source was able to degrade two additions of toluene, however, the rate of degradation slowed significantly after the third addition. For supplemental $(NH_4)_2SO_4$ concentrations less than 1.0 g/ℓ, 18-25 days were required before the toluene was degraded on a regular basis.

As shown in Figure 3, varying the pH of the culture medium greatly affected the degradation of toluene. In the pH range of 2.5-4.0, degradation of the toluene was repeatedly observed, as illustrastrated by the saw-tooth pattern produced from repeated additions of the target chemical. At a pH of 2.0, no toluene degradation was observed. The cell yield was calculated for the cultures used in the pH assay (Table 1). The cell yield varied only slightly among the cultures grown in media with different pH.

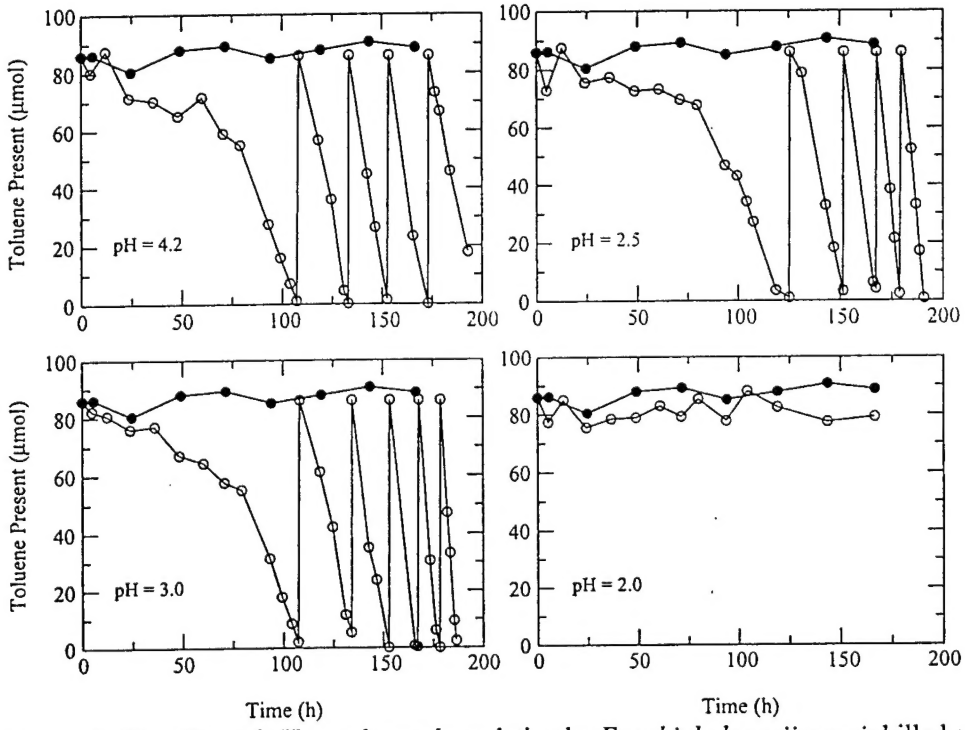

Figure 3. The effect of pH on toluene degradation by *Exophiala lecanii-corni*: killed controls (•) and viable cultures (o).

Table 1. Cell yield and zero order rate constants for *E. lecanii-corni* cultures grown at varying pH.

Culture pH	Cell Yield (g dry weight/g toluene)
4.2	0.61
3.0	0.62
2.5	0.66

Discussion

Exophiala lecanii-corni is able to use many VOCs as its sole source of carbon and energy; however, it is not able to degrade all of the BTEX compounds. Similar results were reported by Cox, et al.[11] In their study, a strain of *Exophiala jeanselmei*, a fungus closely related to *E. lecanii-corni*, was able to degrade styrene, but was unable to degrade ethyl benzene, toluene, or benzene when these compounds were provided as the sole source of carbon and energy. In other studies using fungi to degrade mixtures of TEX[5] and BTEX[12], degradation of all the components was observed, although malt extract was used to supplement growth in the latter study.

In the paint VOC mixture study, the VOC compounds were degraded in the following order: n-butyl acetate, methyl propyl ketone, and toluene. This same order was observed in a bottle study by Kazenski[13], who used a mixed culture containing primarily bacteria. This degradation order may be a result of kinetic and mass transfer differences among the chemicals or due to toxicity effects.[14] Also, aromatic compounds are more difficult to degrade than straight chain compounds, and organisms will typically degrade compounds which provide that greatest amount of energy first.[15]

From the degradation data that was obtained, it was observed that some toluene degradation occurred even without an external nitrogen source, and even small concentrations of external nitrogen allowed for repeated toluene degradation. It has been shown previously that fungi are capable of living in nitrogen-limited environments by utilizing lysed mycelium as nitrogen sources.[16] This property of fungi would be advantageous in biofiltration systems due to the variablity in their operation. Even if nutrients are not regularly supplied to the reactor, operation may not be greatly affected by the fluctuating nutrient availablity.[17]

Although the degradation of toluene slowed when the pH of the culture medium was less than 2.5, *E. lecanii-corni* was still able to readily degrade the target compound under rather acidic conditions. Similar results have been observed in other studies using fungi to degrade VOCs.[8,18–20] Since fungi are capable of living in acidic environments and adjusting to pH fluctuations,[21] fungi may be better suited for use in biofiltration applications, where such shifts in pH are often encountered.

Conclusions

The fungus E. lecanii-corni was shown to degrade a wide variety of VOCs including, toluene, ethyl benzene, methyl propyl ketone, n-butyl acetate, and ethyl ethoxypropionate. Besides using these contaminants as sole sources of carbon and energy, the fungus was also able to degrade mixtures of these compounds. In addition, *E. lecanii-corni* was found to maintain its degradation capabilties under harsh environmental conditions. In culture medium with low nitrogen availability and very acidic pH, sustainable VOC degradation was observed. These preliminary studies indicate that *E. lecanii-corni* would be a feasible organism to use in biofiltration applications due to its ability to degrade a wide range of VOCs, even under harsh environmental conditions.

Acknowledgments

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